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NECROTIC EFFECTS OF THE XENOBIOTICS' ACCUMULATION IN THE CENTRAL NERVOUS SYSTEM OF A CRAYFISH (*ASTACUS LEPTODACTYLUS* ESCHZ.) *

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Abstract

The xenobiotics (Cd, Pb, Hg) accumulate in the central nervous system of the fresh water crayfish. As a result of the effects exercised by them, pathologic structural changes are induced in the cells of the cerebral ganglion - both anterior medial cells in the protocerebrum and olfactory lobe ones in the deutocerebrum - as well as glial cells surrounding the nerve cells. The pathologic changes show the characteristic signs of the hypoxia.

The maximum values of the xenobiotic accumulation develop irrespective of the seasons. On the basis of the mortality data, Cd proved to the most toxic elements from among the heavy metals examined.

Key words: xenobiotics, heavy metals, necrosis, hypoxia, nerve and glial cells

Introduction

Fresh water crayfishes discharge in the aquatic habitat "sanitary" services by living on weakened, ill and perished animals. They are very sensitive to toxic substances issuing from communal, industrial and agricultural sources being mostly responsive for the pollution of standing and running fresh waters. For this reason, the crayfish fauna in the polluted rivers and lakes becomes thinner and species one after the other disappear very often definitely from the habitat (JOHNSON and GENTILE, 1979; SERFŐZŐ et al., 1990a,b; SERFŐZŐ et al., 1992; VALLEE and ULMER, 1972).

Contradictory to the expectations, correlation between the survival or mortality rate of the animals and the quality of water characterized by chemical parameters (Hungarian Standard: MSZ-450.1.89.) in a given habitat could not be demonstrated (SERFŐZŐ et al., 1990a). Indeed, as regards the waters of Eastern Hungary, the mortality rate of crayfishes' populations under experimental conditions remained under 5% in the Ér channel of the branch system of the river Berettyó despite the supportable (IInd class) and often wrong (IIIrd class) water quality, while amounted

* This paper is dedicated to the centennial anniversary of Prof. AMBRUS ÁBRAHAM's birth.

somtimes to 80-100% in the mouth reach of the Keleti Főcsatorna (Eastern Main Channel) as well as in the lakes of Jusztus-Fekete meadow belonging to the Hortobágy National Park characterized mostly by good (Ist class) water quality. It may be supposed that in the background of this phenomenon, the unfavourable, pathobiological effects of accumulations of heavy metals must be searched for (SERFŐZŐ et al., 1992). Considering the discrepancy between the water quality and the mortality rate of crayfishes, we have studied the adaptational disposition in various habitats of a crayfish species being native of the Hungarian rivers. We paid atttention to follow the accumulation of Cd, Pb and Hg in the central nervous system, and examined their effects produced on the structure of the neurons with special regard to the pathological alterations taking place in them.

Material and Methods

Our investigations were carried out on a fresh water crayfish, *Astacus leptodactylus* ESCHZ. The test animals were moved from their natural habitat in the Ér channel being connected with the river Berettyó in Eastern Hungary to new environments choosen according as the reaches in question are exposed to polluting sources of communal, industrial or agricultural character. The lakes of Jusztus-Fekete meadow, a part of the Hortobágy National Park, was regarded as the standard for habitats because of having fresh water protected in theory from polluting effect of all kinds.

The test animals were placed in floating plastic cages, 20 to 25 for each one of the experiment stations. The putting into the new habitats was carried out in the first week of April, the samplings were effected in times depending on the water temperature: in spring and autumn at 12-16, in summer at 22-25 and in winter at 4-6 Celsius. The test material were males of 10-12 cm size.

Except the cerebral ganglion, the whole central nervous system was prepared for the analytical studies. The quantitative determination of the accumulated Cd, Pb and Hg was carried out with GFAAS (Grafite Furnace Atomic Absorption Spectrometry) methods. The cerebral ganglion served for cytopathological investigation. The structural damaging was studied on the anterior medial nerve cell group in the protocerebrum, and on the olfactory lobe nerve cell group in the deutocerebrum, respectively (ABBOTT, 1970, 1971; SANDEMAN, 1982).

The brain sheath was perforated befor the isolation with an injection syringe for then rinsing through the ganglion by a crayfish physiological salt solution (VAN HARREVELD, 1936). Thereupon s. collidine buffered 3% glutaraldehyde solution containing 3mM CaCl2, pH 7.4, was used for prefixing for 3hrs, at 4 Celsius. The samples washed overnight in 0.15 M s. collidine buffer, and postfixed in 1% osmium tetroxide solution buffered with s. collidine for 1 hr, at room temperature. The protocerebrum and deutocerebrum was embedded in Durcupan. The cuttings were made by LKB Ultratome III. Sections stained with uranyl acetate and lead citrate, and viewed in a Tesla BS 540 electron microscope.

For these examinations, the samples were used in which the accumulation of heavy metals showed remarkably high value. It may have been namely expected thet their damaging effects can be more consequently followed and analysed, respectively, in such cases.

Results

1. The increased xenobiotic accumulation in the central nervous system at the spring period of observation

On the sampling fields of the study, in 1987, prominently high accumulation values of xenobiotics in the central nervous system were found in the analysed samples of the spring period. Characterizing, however, the test animals alone taken from the observation posts established in the mouth reach of the Keleti Főcsatorna and in the lakes of the Jusztus-Fekete meadow belonging to the Hortobágy National Park. The quantity of the accumulated Cd, Pb and Hg amounted to 0.46-0.54, 2.04-4.93 and 4.65-6.39 ug/g referring to dry material, respectively, what is equivalent to concentration degrees of 153-180, 227-548 and 9300-12780-fold as compared to the limit values of the Ist class that is good-quality water. The stock of animals set out to this observation post perished almost enterily up to the time of the summer sampling, the mortality rate came to 93 and 100%, respectively.

The xenobiotic load leaves lasting marks on the anterior medial nerve cell group of protocerebrum. Swollen, rounded or collapsed, large-sized spaces develop from necrotized mitochondria and Golgi's vesicles in the cytoplasm, being surrounded by tightly linked glial cell processe. The endoplasmic reticulum becomes fragmented and disintegrated into small-sized vesicles. Pycnosis of the nucleus indicates its necrosis (Figs 1 and 2). Intact cell organelles are hardly or not et all to be found in the neurons.

Changes like those in the anterior medial nerve cells take place in the cytoplasm of the olfactory lobe nerve cells belonging to the deutocerebrum, too, with the difference, however, that nuclear pycnosis does not develop in them, while the structural changes caused by necroses in the cell organelles seem to be still more dramatic, because their relatively small cytoplasm is almost totally filled up by the swollen organelles (Fig. 3).

The pathologic structural changes in the neurons cover the axons, too. Local membrane hyperplasia and degeneration as well as the numerical decrease and agglutination, respectively, of the neurotubules may to be observed in the axons - surrounded by glial cell processe - of the anterior medial nerve cell group (Figs 4 and 5).

The glial cell processes, separating the neurons from one another while sticking to them, constitute loose layers. Lines of vesicles arranged like strings of pearls may be seen in the processes by filling up entirely their interior (Figs 4 and 5). Their continuity is broken here and there by the occurrence of residual bodies of various size. The membrane hyperplasia and degeneration observed in the necrotizing neurons appear repeatedly in the membranes of the processes of glial cells, too (Figs 2 and 4).

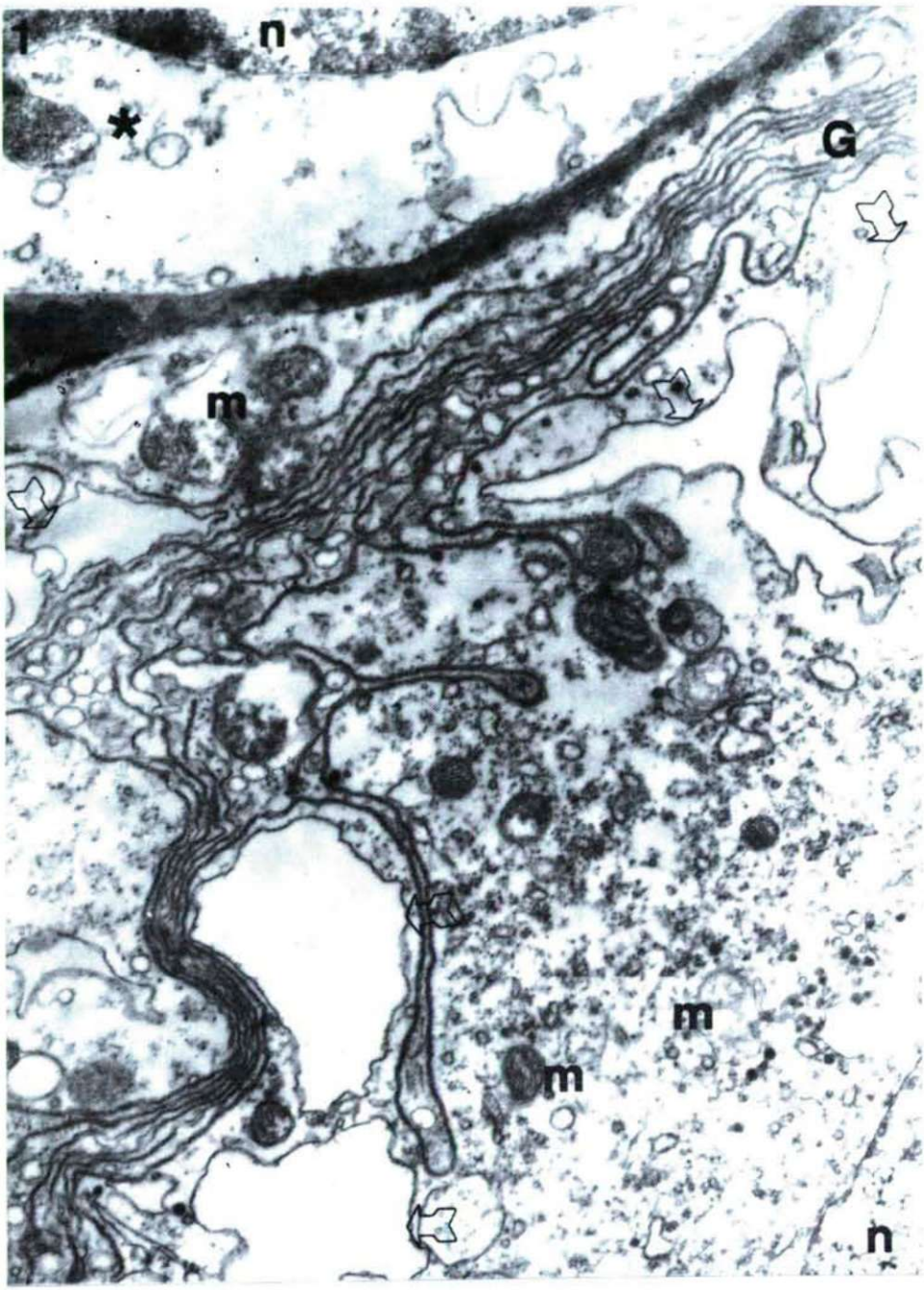

Fig. 1. Cell damaging with pycnotic nucleus (•), necrotized cytoplasm, and swollen and collapsed Golgi vesicles (white arrows) in the anterior medial nerve cell group x21000. Abbreviations and Legends: a = axon; G = glial cell processes; ga = Golgi apparatus; m = mitochondrion; n = nucleus; rb = residual body.

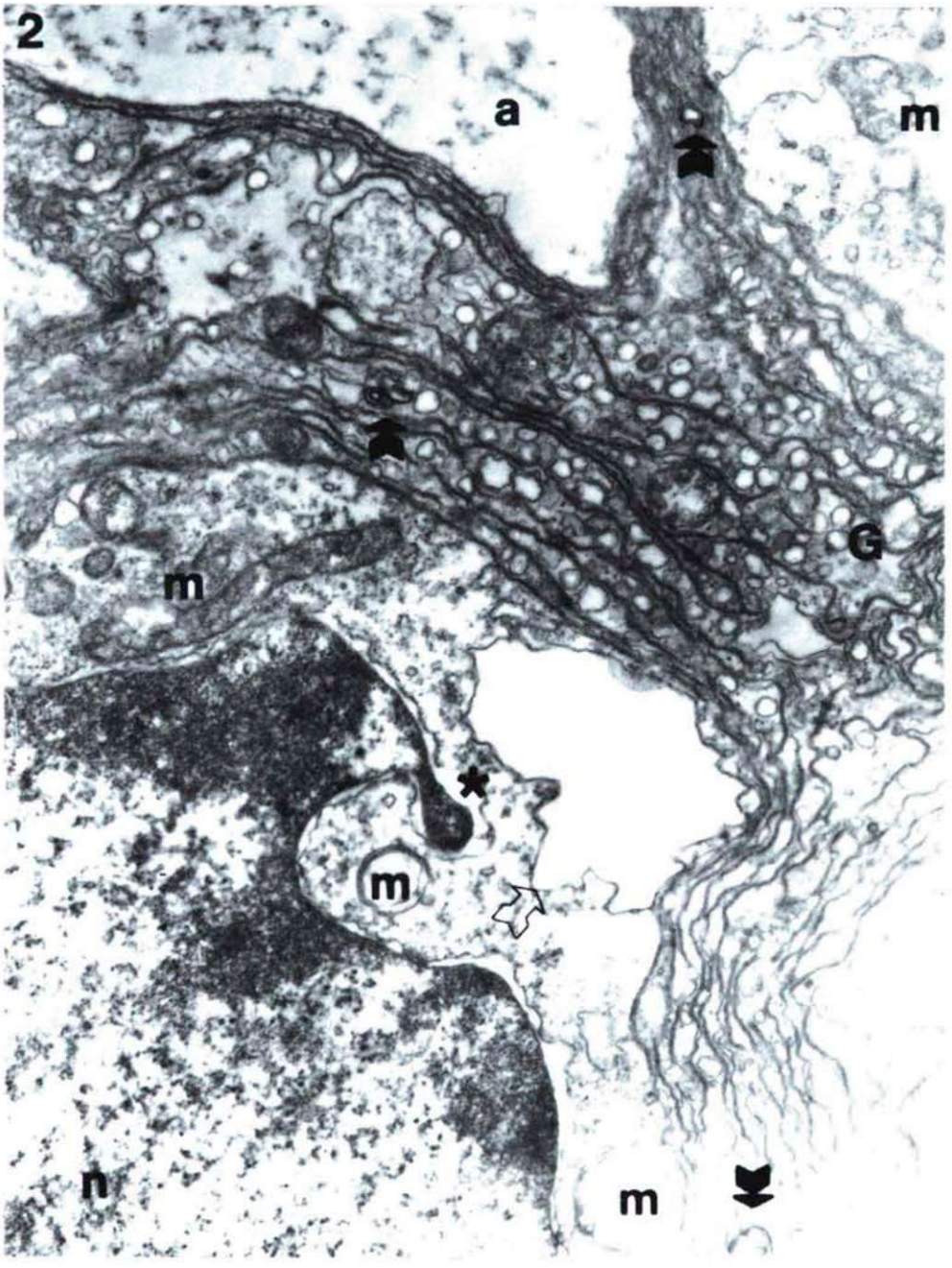

Fig. 2. Increased development of vesicles and local membrane damaging (black arrows) in the glial cell processes. There is pycnotic nucleus (*) and polygonal Golgi vesicle in the anterior medial nerve cell (white arrow). x21000

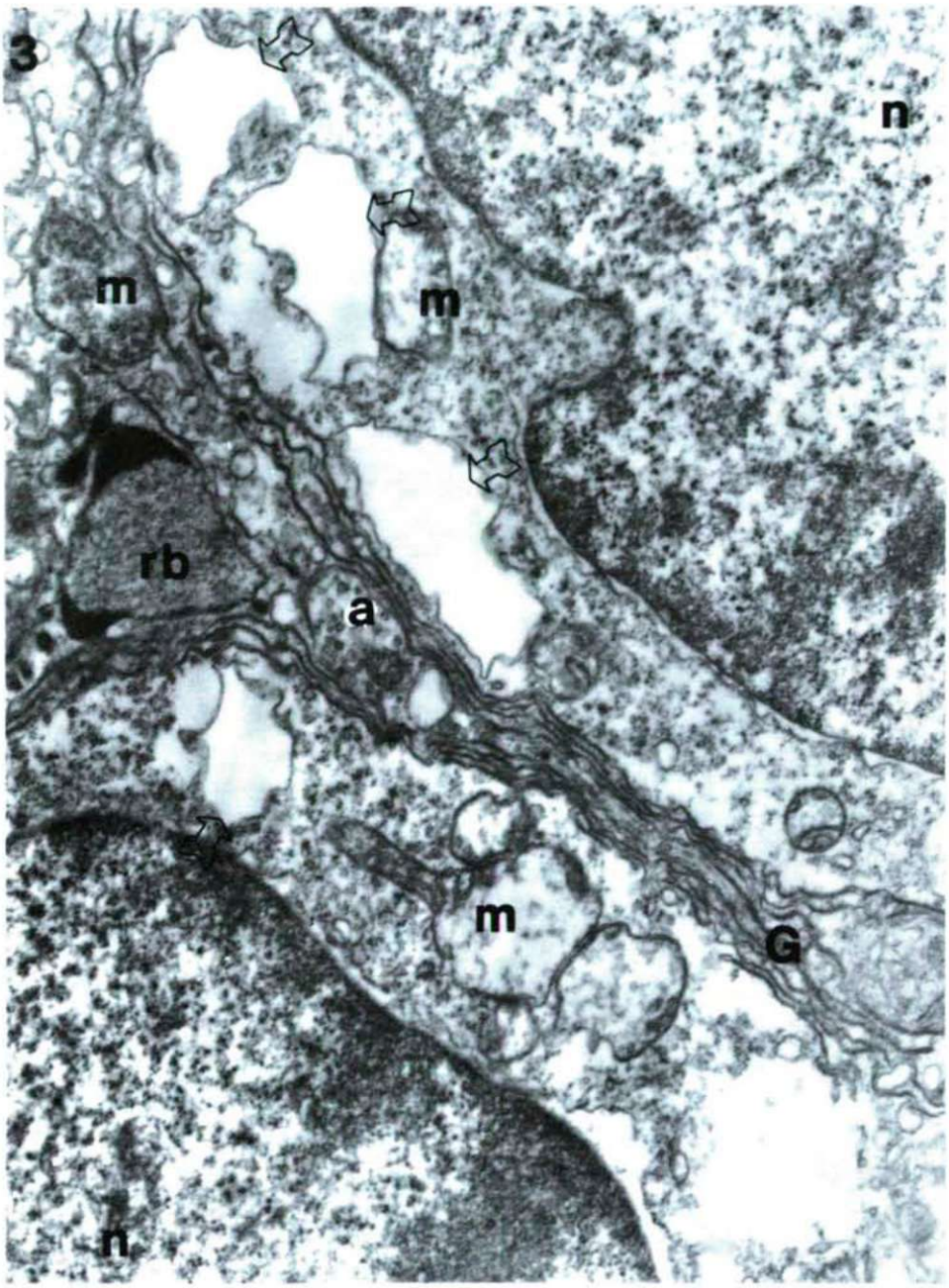

Fig. 3. Swollen and polygonal Golgi vesicles in the deutocerebral olfactory lobe neurons. (White arrows) x21000

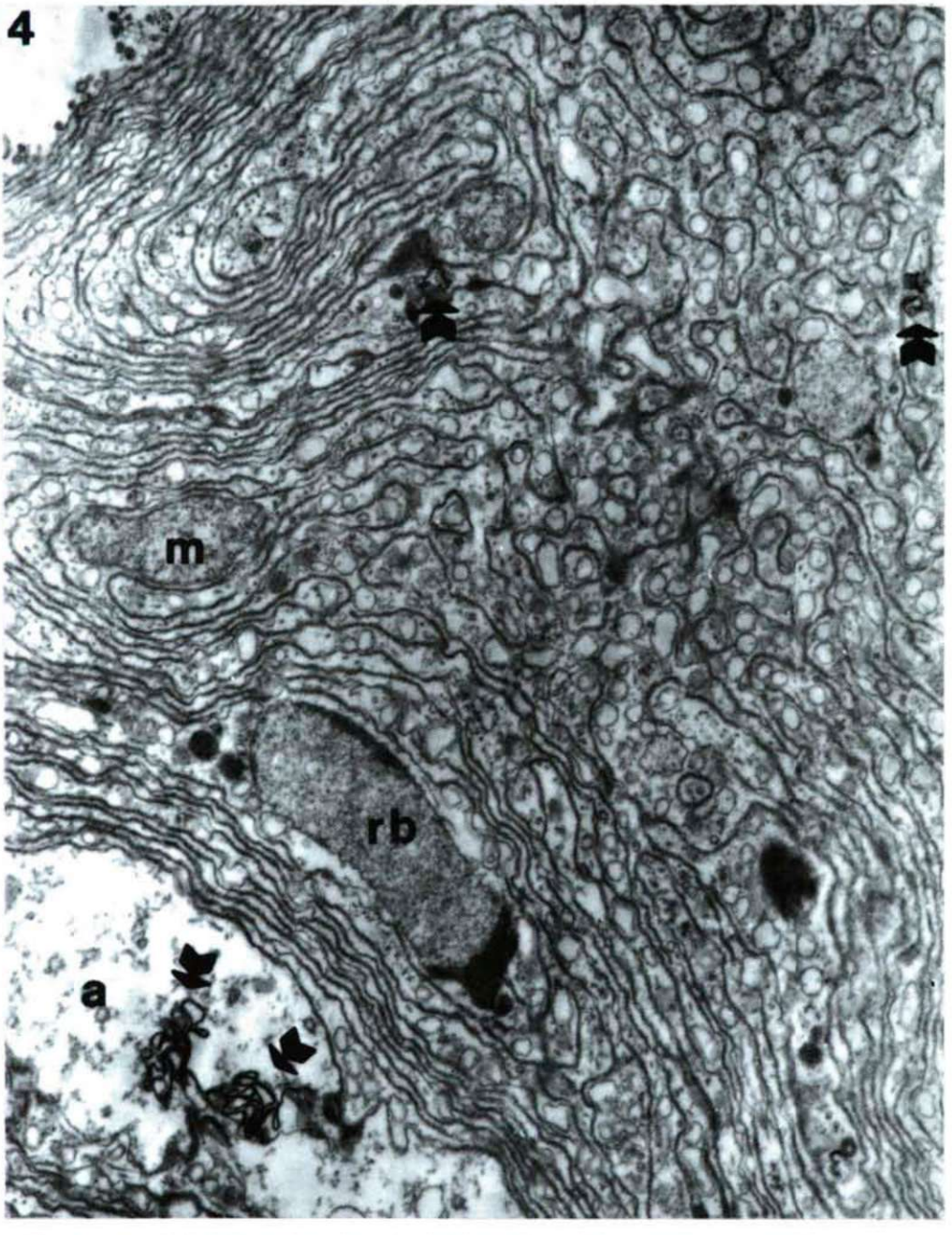

Fig. 4. Axon and glial cell membrane damaging in the anterior mediel nerve cell cell group. (Black arrows) x21000

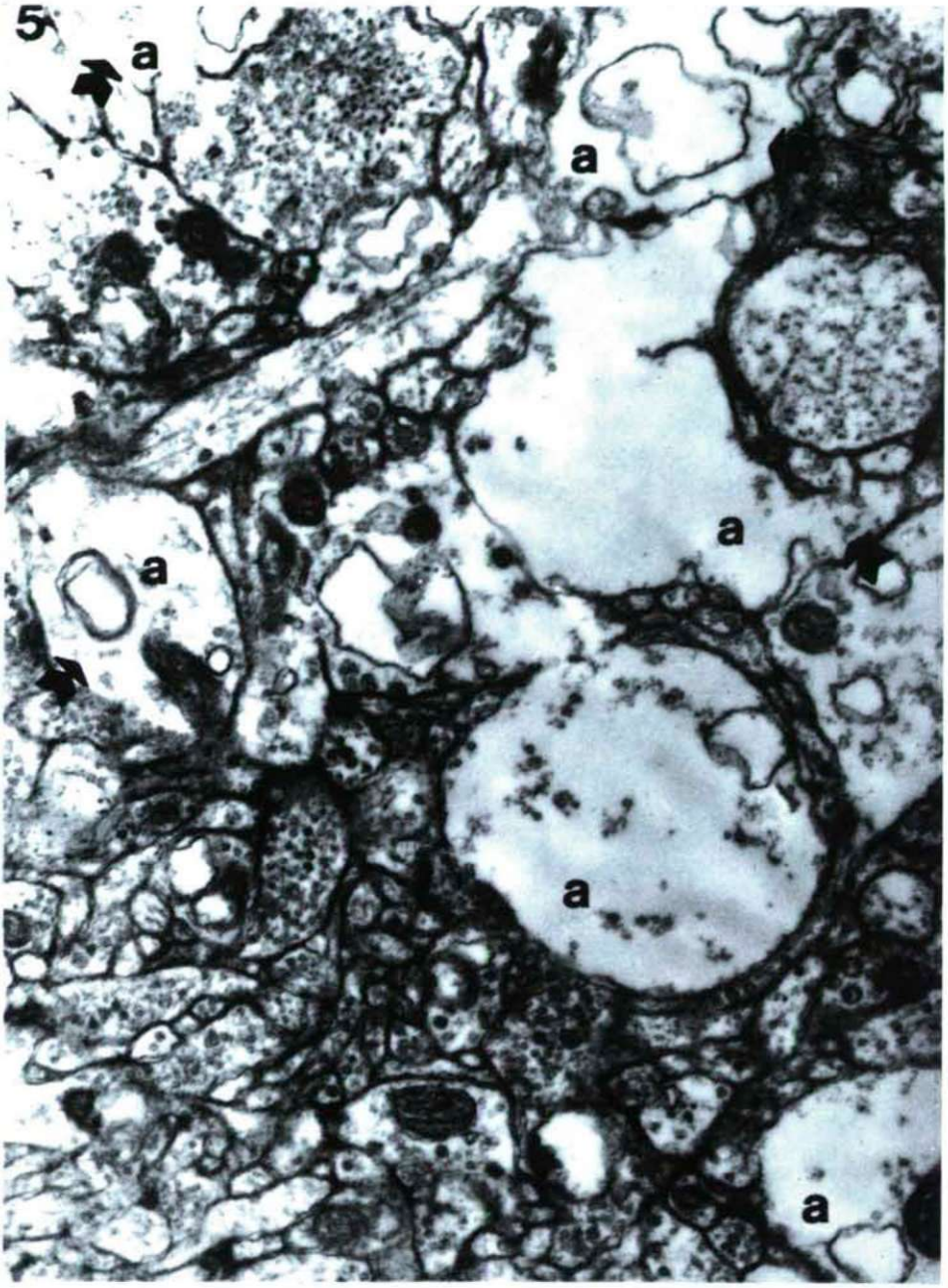

Fig. 5. Axon damaging between the area of anterior medial and olfactory lobe nerve cell group. (Black arrows) x24000

2. The increased xenobiotic accumulation in the central nervous system during the summer period of the observation

Among the samples examined in the summer period of 1988, we found cases showing prominently high values of xenobiotic accumulation. The samples in question set in the river Berettyó. The quantity of Cd, Pb and Hg was found to be 0.08, 8.07 and 1.4 ug/g referring to dry material, respectively, what means enrichment to 27, 897 and 2800-fold as much as compared to the limit of the Ist class that is good-quality water. The mortality rate in the sampling period, with the given xenobiotic load, came to 41%.

The structure of the anterior medial nerve cell group of the protocerebrum was influenced by the accumulated elements efficiently in thise cases, too. The damaging effect, however, was not so generally extended on all neurons and so drastic as in the spring period's samples examined in 1987. The most apparent pathologic alterations develop in the mitocondria: disorganized internal membrane and swollen organelle. The Golgi's vesicles are characterized by oedema, after having swollen they collapse (Fig. 6). It comes to fragmentation of the endoplasmic reticulum, to nuclear pycnoses, however, it dosn't. The permanent presence of mitochondrial and Golgi's swollen vesicles occupying large spaces in the cytoplasm points, however, that phenomena remaining not local and being able to induce extended erosions may take place at any time in the intracellular spaces.

The influence of the given xenobiotic load on the structure of the olfactory lobe neuron group in the deutocerebrum proved to be slight, the cell organelles are not damaged in a remarkable degree. Oedematous status did not develop, and the incidence rate of axon degeneration is negligible, too. (Fig. 7.)

The xenobiotic accumulation had no slightly appearing effect damaging glial cells in either of the two neuron groups. In the glial cell processes of the samples investigated, it came neither to an increased forming of vesicles, nor to the development of oedema and to the loosening of the processes. The linking between glia and nerve remained normal.

3. The increased xenobiotic accumulation in the nervous system at the autumnal term of the experiments

On the observational posts placed in the river Berettyó, increased xenobiotic accumulation in the central nervous system of the test animals was found not only in the summer but also in the autumnal period of 1988. The quantity of Cd, Pb and Hg was 0.028, 5.3 and 1.4 ug/g referring to the dry material, respectively, what means concentration of 9, 589 and 2800-fold as compared to the limit of the Ist class that is good-quality water. At the term of the sampling, the animals were healthy, none of them perished.

Excepting nuclear pycnosis, all the structural changes previously described may have been observed in the anterior medial nerve cells group of the protocerebrum (Fig. 8), that is mitochondrial degeneration, development of large-sized, oedematous

vesicles, fragmentation of the endoplasmic reticulum in the cytoplasm. However, the damaging effects on the mentioned cell organelles do not reach the critical measure that would result in the death of the nerve cells.

In the cell group of the deutocerebral olfactory lobe, signs of nuclear necrosis may not be observed. If it still occured, did not rise above the measure being in accordance with a hypertrophic activity under otherwise normal circumstances (Fig. 9).

The glial cell processes surrounding the nerve cells are regular. Vesicles may be found in them but sporadically, oedema does not develop at all. Their structure indicates the normal state for as much as their linking up to the nerve cells is free from pathologic alterations, e.g. from hyperplasic membrane areas, too.

Discussion

Having analysed the water quality of fresh waters in Eastern Hungary, the river Berettyó and its branch system, the lakes of Jusztus-Fekete meadow in the Hortobágy National Park, we found not a single parameter used to qualification of waters, e.g. oxigen, ammonia, pH, temparature, detergents, oils, etc, the acute and chronic change of which would have furnished informations about the rate, place and time of mortality of the crayfish (SERFŐZŐ et al., 1990 a,b). The discrepancy appearing between the changes in the quality of water and the mortality rate of the experimental animals may be resolved, according to our investigations, with knowledge of the accumulation of xenobiotics in the organism of the animals. We have established that the Cd already in a concentration of 100 ng/g increases markedly the mortality rate, and by or over a loading of 300 ng/g, respectively, induce the almost entire perishing of the stock of animal placed in the experimental stations (SERFŐZŐ et al., 1991).

From the present examinations, it is evident that the efficiency of Cd is not or not strikingly, respectively, increased by the accumulation of Pb and Hg. The Cd and Hg contents of the samples of the summer and autumnal terms are small as compared to that in the spring samples. It seems to be characteristic that despite the hihg Pb content in the summer and autumnal samples, the rate of survival becomes better, that is the mortality rate decreases.

The maximum values of the xenobiotics' accumulations may not be connected with one of terms in the year influencing the vital processe of the animals (SERFŐZŐ, 1990c). These namely, may have been observed anywhere on the field of sampling and at any term. Consequently, the phenomenon may not be attributed mainly to causes arising from the feed but to changes taking place in the aquatic habitat. A former observation, the increase of Mn content of the central nervous system retardes the damaging effects of Cd, Pb and Hg, points to this, too (SERFŐZŐ et al., 1990b).

The xenobiotics influence damaging the structure of nerve cell groups of the cerebral ganglion. This appears most strikingly in the necrosis of the interior membrane of the mitochondria. Moreover, it comes to development of abnormal

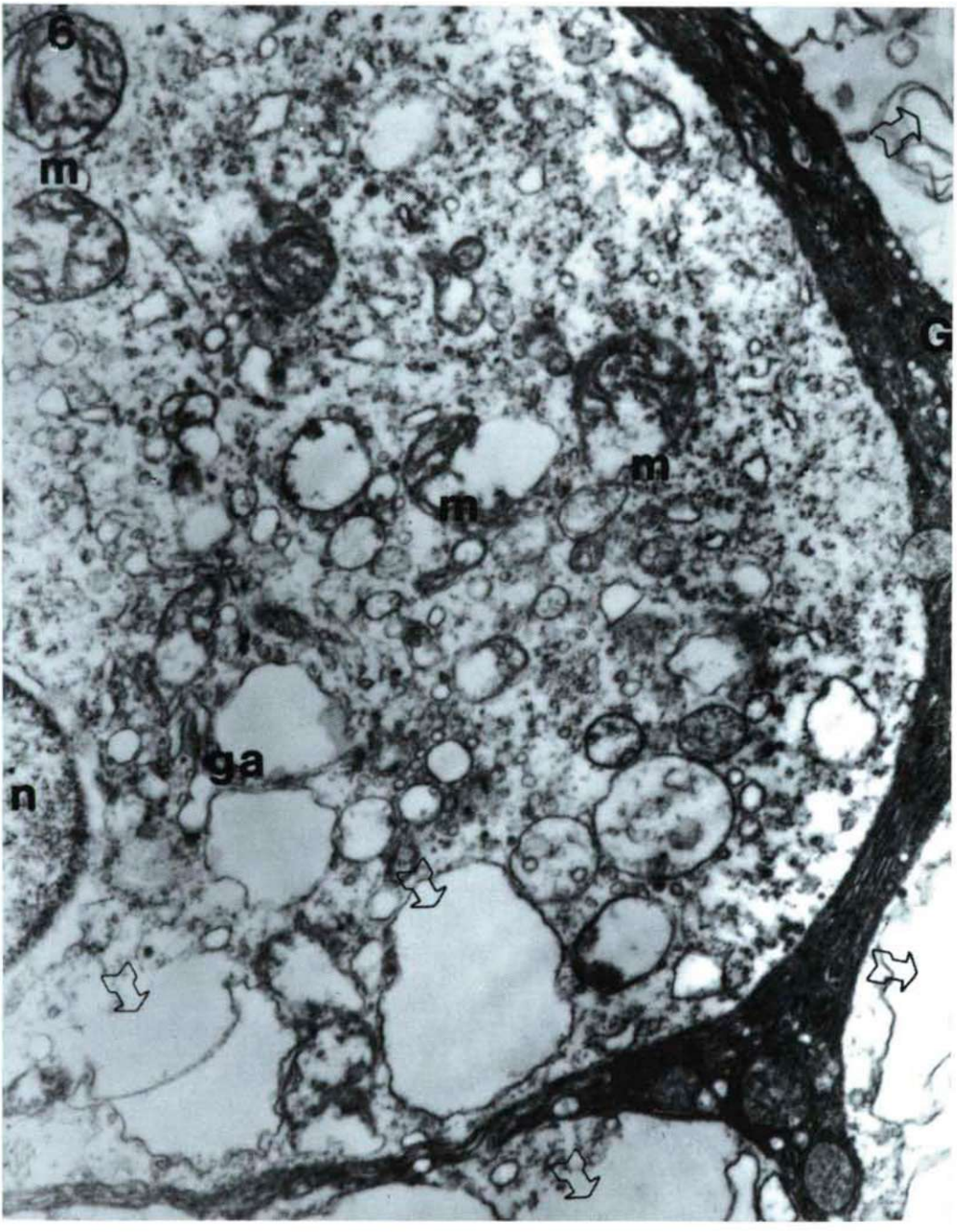

Fig. 6. Slighty damaging nerve cell of the anterior medial nerve group with swollen and collapsed Golgi vesicles. (White arrows) x21000

Fig. 7. Mitochondria damaging and formation of myelin bodies in the olfactory lobe nerve cell group. (Black arrows). x21000

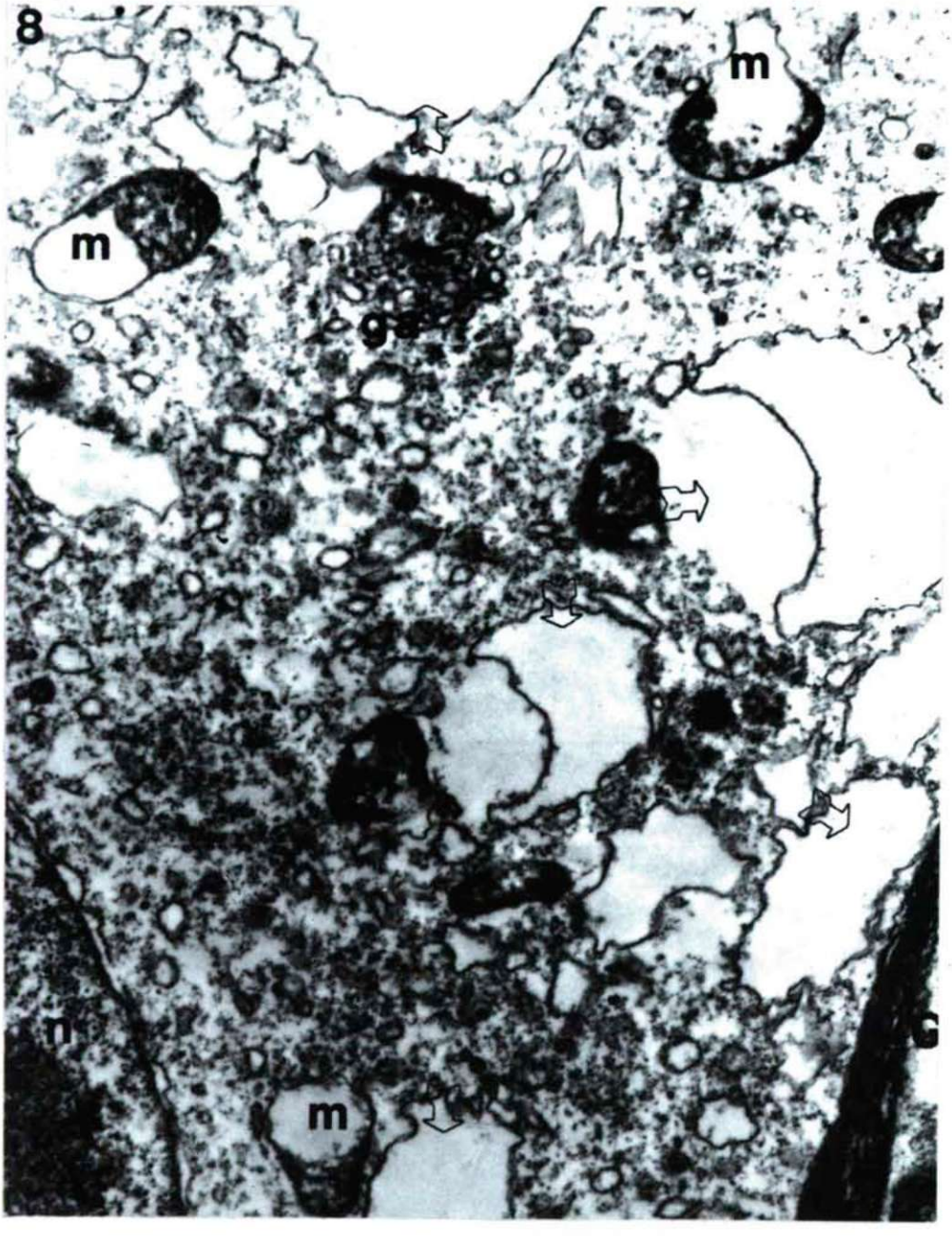

Fig. 8. Increased formation of swollen and polygonal Golgi vesicles in the anterior medial nerve cell. (White arrows) x21000

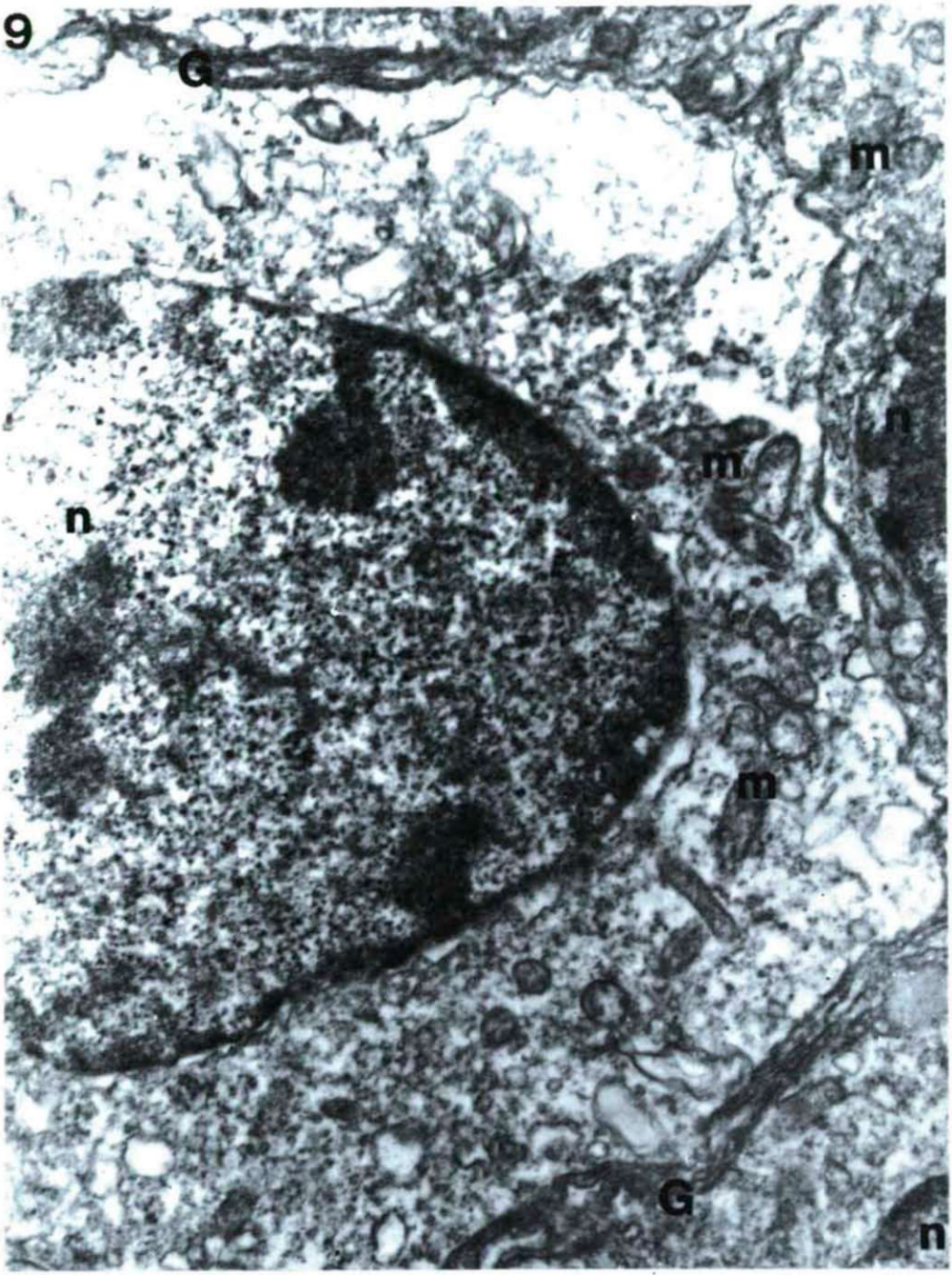

Fig. 9. Slightly hypertrophysed nerve cell in the olfactory nerve cell group. x21000

Golgi's vesicles, fragmentation of endoplasmic reticulum and nuclear pycnosis, too. Membrane degenerations appear mainly in the axons. During the pathologic processes, the cell organelles are swollen, but among the Golgi's vesicles, collapsed, polygonal forms are to be observed, too. The syndrome described points to hypoxia (CONSTATINIDES, 1984; GOYER, 1991). Considering, however, that in the nerve cell groups examined, not all the cells show strikingly marked structural damages, we have to suppose that either also other factors may have a share in the process or the susceptibility of the damaged nerve cells is the source of the difference. The xenobiotic accumulation does not allow to remain intact the structure of the glial cell processes surrounding the nerve cells, too. The processes fill with vesicles, become swollen, degenerated areas are to be found in their membranes. There is a close relationship between the state of the glial cell processes and the pathologic changes appearing in the nerve cells. In all the cases when the glial cell loosens the linking between glia and nerve, this happens during the increased formation and swelling of vesicles, the consequences of the xenobiotic accumulation appear in the nerve cells more strikingly, and conversely. (SERFŐZŐ et al., 1991; SERFŐZŐ et al., 1992).

Summary

The central nervous system of the crayfish is able to accumulate xenobiotics in considerable quantities. In the manmade habitats set up in the rivers and lakes of Eastern Hungary. The concentration degree of Cd, Pb and Hg was 9-180, 227-897 and 2800-1278-fold, respectively, as compared to the limit values characteristic of the Ist class that is good-quality water. The maximum values of the accumulation developped irrespective of the seasons. It might not be drawn a parallel between the fact of accumulation and a vital functions of the animals. On the basis of the mortality data, Cd proved to be the most toxic from the heavy metals examined.

As a result of the xenobiotics' accumulation, the fine structure of the nerve cells has to be suffer changes. These include almost every cell organelles by inducing nuclear pycnosis, mitocondrial disorganisation, abnormal development and collapse of Golgi's vesicles, fragmentation of endoplasmic reticulum and alterations in the membrane of the axons.

Changes take place in the glial cell processes, too: increased development of vesicles including almost the whole system of the processes, emergence of residual bodies and membrane degenerations.

The degenerative alterations taking place in the fine structure of both the nerve and glial cells may be brought in overlapping with the degree of the xenobiotic accumulation. The symptomes are typic manifestational forms of the hypoxia.

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